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A NOVEL CLASS OF Na^+ AND Ca^{2+} CHANNEL DUAL BLOCKERS WITH HIGHLY POTENT ANTI-ISCHEMIC EFFECTS

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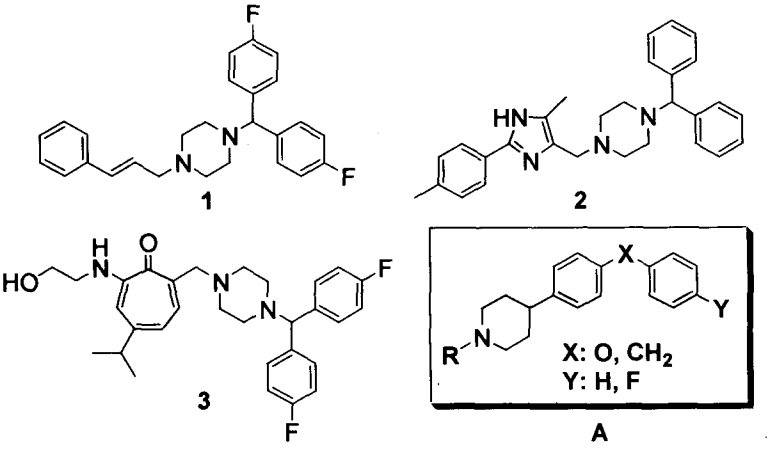
Abstract: A series of novel arylpiperidines (**4a-d**) which have highly potent blocking effects for both neuronal Na^+ and T-type Ca^{2+} channels with extremely low affinity for dopamine D2 receptors were synthesized. Among these compounds, 1-(2-hydroxy-3-phenoxy)propyl-4-(4-phenoxyphenyl)-piperidine hydrochloride (**4c; SUN N5030**) exhibited remarkable neuroprotective activity in a transient middle cerebral artery occlusion (MCAO) model.

Ca^{2+} overload, characterized by a rise in intracellular Ca^{2+} concentration to a pathological level due to ATP depletion followed by the failure of an intracellular ion homeostasis, is directly connected to cell death or damage caused by ischemia.[1] It has recently been demonstrated that the activation of Na^+ and Ca^{2+} channels is extensively involved in the Ca^{2+} overload pathway and the accumulation of intracellular Na^+ ions is rapidly converted into Ca^{2+} overload by the reverse operation of Na^+/Ca^{2+} exchange mechanism.[1, 2] Although only a few types of Na^+ and/or Ca^{2+} channel blockers including flunarizine[2b, 3] (**1**), lifarizine (RS-87476)[4] (**2**) and U-92032[5] (**3**), that have in common a diphenylmethylpiperazine moiety, have been reported as Ca^{2+} overload blockers[2a, 6] and shown to protect neuronal cell death in animal models, there is a need for the development of more potent compounds with reduced affinity for dopamine D2 receptors in order to avoid clinical risk of extrapyramidal side effects.[7] In this communication, we report the synthesis of a structurally novel class of arylpiperidines (**4a-d**) having the general formula (**A**) that exhibit highly potent blocking effects for both neuronal Na^+ and T-type[8] Ca^{2+} channels with extremely low affinity for dopamine D2 receptors. The effects of **4a-d** in *in vivo* models are also described.



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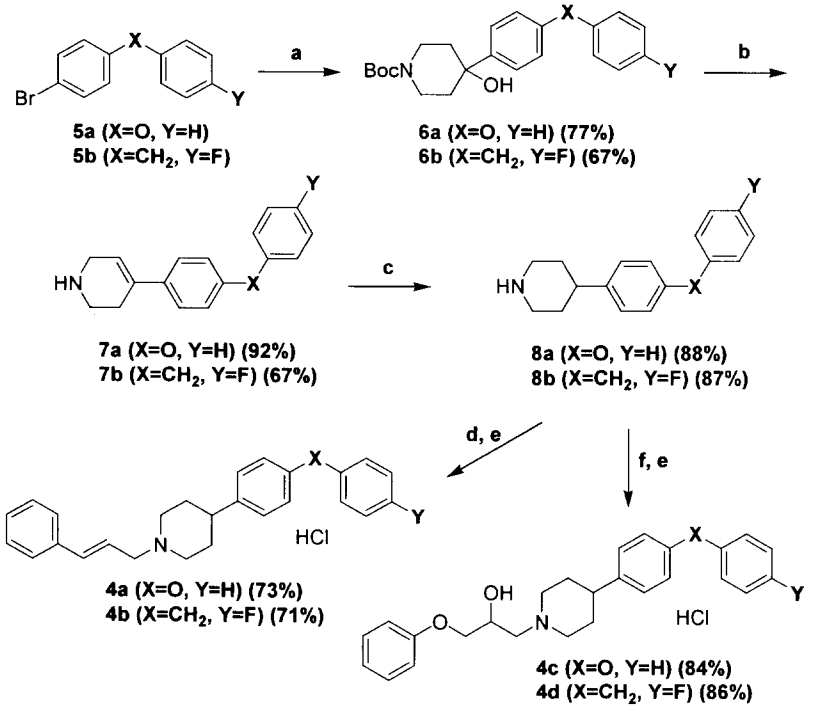
Chemistry

Compounds **4a-d** were prepared using the pathway shown in Scheme 1. Treatment of N-*tert*-butoxycarbonyl-4-piperidone with the Grignard or lithium reagent prepared from the corresponding aryl bromides **5a,b** in a conventional manner gave, **6a** (77%) and **6b** (67%), respectively.[9] Deprotection of the Boc group in **6a,b** by exposure to trifluoroacetic acid proceeded with dehydration of *tert*-hydroxy group gave the tetrahydropyridine derivatives, **7a** (92%) and **7b** (67%), respectively. Hydrogenation of **7a,b** in the presence of a catalytic amount of Pd-C in methanol yielded the requisite arylpiperidines, **8a** (88%) and **8b** (87%), respectively. Reactions of **8a,b** with cinnamyl bromide in the presence of triethylamine in acetonitrile or with phenyl glycidyl ether in 2-propanol followed by treatment with ethanol saturated with hydrochloric acid gave **4a-d** in 71-86% yields after recrystallization from ether/methanol. When chiral phenyl glycidyl ethers were employed in the final step, both enantiomers of **4c,d** with respect to the secondary alcohol moiety could be obtained in similar chemical yields with >98% ee.

Scheme 1[a]



[a] **(a) (1) Mg, THF or n-BuLi, THF, -20 °C, (2) N-*tert*-butoxycarbonyl-4-piperidone, 0 °C, 1h; (b) TFA-CH_2Cl_2 (1:1), r.t., 12h; (c) H_2, cat.Pd-C, MeOH, r.t., 12h; (d) cinnamyl bromide, Et_3N, MeCN, 80 °C, 2h; (e) HCl-EtOH; (f) phenyl glycidyl ether, i-PrOH, reflux, 2h.**

Results and Discussion

The effects of a series of the synthetic compounds **4a-d** for Na^+ channels were evaluated by inhibitory action on veratridine-induced depolarization in rat cerebrocortical synaptosomes using the voltage-sensitive

fluorescent dye Rhodamine 6G.[10] The effects of **4a-d** on low-threshold (T-type) Ca^{2+} currents in primary cultured rat cerebrocortical neurons were examined using whole-cell voltage-clamp recording technique.[3c] As shown in Table 1, **4a-d** were found to block both Na^+ and T-type Ca^{2+} channels with potency greater than or equal to flunarizine (**1**) which was adopted as a reference standard. Compounds **4a-d** showed concentration-dependently block of T-type Ca^{2+} currents induced by a depolarizing pulse to –40 mV from holding potential (V_H) of –100 mV. The dopamine D2 receptor binding affinity was assessed using [^{3}H]-racoplide as a ligand binding to rat striatum membranes.[11] In remarkable contrast to the potent activity on Na^+ and T-type Ca^{2+} channels, **4a-d** exhibited extremely low affinity for dopamine D2 receptors. Surprisingly, **4d** practically lost its binding affinity for dopamine D2 receptors. These differences clearly demonstrate that **4a-c** possess structural features distinctly different from those of flunarizine (**1**) and its analogues. The racemate **4c** and the both enantiomers, **(S)-4c** and **(R)-4c**, could not be discriminated with respect to the potency and selectivity in these assays.

Table 1. Biological Activity of **4a-d**

entry	IC_{50} (μM)			anticonvulsant effects in DBA/2 mice[d]
	anti-veratridine[a]	T-type Ca^{2+}currents[b]	D_2[c]	ED_{50} (mg/kg; i.p.)
4a	0.32	0.8	2.68	4.2
4b	0.19	0.6	3.38	2.2
4c	0.22	3.5	4.64	5.0
(S)-4c	0.13	2.0	4.34	2.5
(R)-4c	0.12	2.7	4.08	2.5
4d	0.36	0.8	>10	7.5
1	0.29	2.2	0.228	6.4

[a] See ref.10. [b] See ref.3e. [c] See ref.11. [d] See ref.12.

Next, we investigated the effects of **4a-d** on audiogenic seizures in DBA/2 mice to confirm their *in vivo* activity and permeability into brain.[12] These compounds **4a-d** proved to exhibit potent anticonvulsant effects following systemic (ip) administration with ED_{50} values as shown in Table 1. We also assessed the neuroprotective activity of **4a-d** on transient MCAO[13] for 60 minutes in rats by measuring peripheral type benzodiazepine binding site[3b] (PTBBS) densities in ipsilateral cortical and striatal homogenates as a quantitative index for neuronal damage 10 days after reperfusion. The each compound was administered immediately after both MCAO and reperfusion (each 3mg/kg, iv). Consequently, **4a,c** significantly reduced PTBBS levels by 47.5 and 65.8%, respectively (*$p<0.05$ vs. vehicle), while **4b,d** effected only minor reductions. In particular, **4c** showed a 1.7 fold higher potency but had 1/20 the affinity for dopamine D2 receptors as compared with flunarizine (**1**), that reduces PTBBS levels by 37.9% (*$p<0.05$) in this MCAO model. These results indicate that **4a,c** have a pronounced neuroprotective efficacy against neuronal damage induced by transient focal ischemia in rats. Interestingly, both compounds **4a,c** at the effective doses had no effects on systemic blood pressure and heart rate in anesthetized rats. In support of this, **4a,c**

were found to cause reversible inhibition of Na^+ currents in a concentration- and voltage-dependent manner on Na^+ currents in primary cultured rat cerebrocortical neurons using whole-cell voltage-clamp recording technique. The IC_{50} values of **4a,c** obtained at V_H of −100 mV were more than 10 μ M and 5 μ M, respectively, whereas these were lowered to 1.4 μ M and 0.7 μ M, respectively, at V_H of −70 mV. It should be noted that the markedly enhanced voltage dependency promises of event specific inhibition for ion channels without primary haemodynamic adverse effects.[2]

In conclusion, we described the synthesis and biological evaluation of a novel class of arylpiperidines **4a-d** that show not only highly potent blocking effects for both neuronal Na^+ and T-type Ca^{2+} channels but also extremely low affinity for dopamine D2 receptors. The compound **4c (SUN N5030)** has a desirable pharmacological profile and would be useful in the alleviation and treatment of ischemic diseases.[1, 2] The effects on other subclasses of Ca^{2+} channels and the structure-activity relationships of this series of compounds will be reported in subsequent communications.

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